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(54) **Use of L-carnitine and alkanoyl L-carnitines in the storage of blood for transfusions and stabilizing solutions containing them**

Verwendung von L-Carnitin und Alkanoyl L-Carnitine zum Bewahren von Transfusionsblut, und sie enthaltenden Stabilisierungslösungen

Utilisation de L-carnitine et alkanoyl L-carnitines dans le stockage de sang pour les transfusions, et inlieux de stabilisation les contenant

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Description

The present invention consists in a new non-therapeutic use of L-carnitine, alkanoyl L-carnitines and their pharmacologically acceptable salts as preservative agents for blood for transfusions.

The present invention also consists in new stabilizing solutions for the storage of blood containing L-carnitine, alkanoyl L-carnitines or their pharmacologically acceptable salts.

What is meant by alkanoyl L-carnitines are acetyl, propionyl, butyryl, isobutyryl, valeryl and isovaleryl L-carnitine. Hereinafter, for reasons of simplicity, we shall refer only to L-carnitine, in the understanding, however, that the description also applies to the above-mentioned alkanoyl L-carnitines and their pharmacologically acceptable salts.

As is well known, L-carnitine is necessary for the translocation of fatty acids within the mitochondria where beta-oxidation takes place.

Various uses of L-carnitine are known, but all of these are of a therapeutic nature. For instance, L-carnitine is used in the cardiovascular field for the treatment of acute and chronic myocardial ischaemia, angina pectoris, heart failure and cardiac arrhythmias.

In the nephrological field, L-carnitine is administered to chronic uraemics undergoing regular haemodialytic treatment to combat myasthenia and the onset of muscular cramps.

Other therapeutic uses have to do with the normalization of the HDL:LDL+VLDL ratio and total parenteral nutrition. There is, however, no relationship between the known therapeutic uses of L-carnitine mentioned previously and the use envisaged in the present invention.

It is well known that the essential factors for good storage of blood in the liquid state are the temperature and the composition of the stabilizing solution.

The temperature must be such as to allow a reduction of the metabolic activity of the erythrocytes without damaging them. The optimal temperature is $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

The stabilizing solutions must be able to make the blood unclottable, to reduce the glycolytic activity of the red blood cells and, at the same time, permit such activity by providing an adequate substrate.

The efficacy of a stabilizing solution is assessed by observing both the alterations arising in the erythrocytes *in vitro* and their survival *in vivo*, after variable periods of storage at optimal temperature.

The alterations to the erythrocytes *in vitro* can be checked by evaluating the amount of haemoglobin released by the erythrocytes, their osmotic and mechanical fragility, the changes in their shape and volume and the chemical changes they undergo.

The stabilizing solutions used to date do not allow good storage of blood for more than 14 to 21 days.

For instance, if the blood is collected in ACD (citric acid-sodium citrate-dextrose), one of the most widely used stabilizing solutions in the past, and transfused

after 14 or 21 days of storage, the *in vivo* survival rates of erythrocytes 24 h after transfusion are 90 and 80%, respectively; it is also well known that red blood cells that remain in circulation 24 h after transfusion have a survival rate equal to that of fresh blood.

During storage, erythrocytes undergo alterations with formation of spherocytes and burr cells. The erythrocytes swell and lose potassium and haemoglobin, which then increases in the plasma. At the same time there is a reduction in 2,3-DPG (2,3-diphosphoglycerate) and thus an increase in the affinity of haemoglobin for oxygen, which is released to tissues in smaller amounts.

The alterations of erythrocytes stored in ACD may be at least partly corrected by adding phosphate to the stabilizing solution. Thus CPD (citrate-phosphate-dextrose) solutions have come to be used for the storage of blood and are now the ones most commonly employed. The addition of phosphate gives rise to the maintenance of a higher level of 2,3-DPG and thus a lower affinity of haemoglobin for oxygen. However, the *in vivo* survival of erythrocytes stored in CPD is little better, if not indeed identical to that of erythrocytes stored in ACD.

It has now been found that the addition of L-carnitine or of one of its pharmacologically acceptable salts to the usual stabilizing solutions for the storage of blood for transfusions has the effect of dramatically improving *in vitro* survival of erythrocytes and of reducing the formation of spherocytes and burr cells and the loss of haemoglobin in the plasma. As a result of these beneficial effects, the period of good storage of blood for transfusion purposes is more than doubled compared to traditional solutions.

Thus, the invention described herein consists in the use of L-carnitine and its pharmacologically acceptable salts for the production of stabilizing solutions for the storage of blood for transfusions.

Viewed from a different angle, the invention also consists in stabilizing solutions for the storage of blood for transfusion characterized by the fact that they contain L-carnitine or one of its pharmacologically acceptable salts.

What is meant by pharmacologically acceptable salts of L-carnitine, apart from the L-carnitine internal salt, is any L-carnitine salt with an acid which does not give rise to unwanted side effects. These acids are well known to pharmacologists and to experts in pharmacy.

Non-exclusive examples of such salts are chloride, bromide, crotonate, aspartic acid, acid citrate, acid phosphate, fumarate and acid fumarate, maleate and acid maleate, acid oxalate, acid sulphate, glucose phosphate, tartrate and acid tartrate.

These solutions are characterized by the fact that they contain 0.5-10.0 mM/L, and preferably 4-6 mM/L of L-carnitine or an equivalent amount of one of its pharmacologically acceptable salts.

An example of a stabilizing solution according to the invention is composed of:

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|---------------------------------|-------------|
| Glucose | 80-120 mM/L |
| Mannitol | 40-60 mM/L |
| K ₂ HPO ₄ | 24-28 mM/L |
| KH ₂ PO ₄ | 12-16 mM/L |
| Potassium citrate | 15-20 mM/L |
| L-carnitine, internal salt | 4-6 mM/L |

The efficacy of L-carnitine in the use envisaged in this invention is verified by numerous studies, one of which is reported here below with reference to the attached diagrams, where:

- Fig. 1 is a graph representing the osmotic fragility of erythrocytes as a function of storage time (expressed in weeks);
- Fig. 2 is a graph representing the haemoglobin concentration (in mg/dL) in the storage medium as a function of storage time (expressed in weeks);
- Fig. 3 is a graph representing the percentage of burr cells in the storage medium as a function of storage time (expressed in weeks).

Admitted to this study were volunteers who were habitual blood donors and who were perfectly healthy at the time the blood samples were taken. The blood samples were collected in special quadruple bags normally used for blood storage (Baxter, Fenwal Division, La Châtre, France) containing CPDA-1 (CPDA-1 composition: 110 mM glucose; 55 mM mannitol; 25.8 mM K₂HPO₄; 14.7 mM KH₂PO₄; 17.9 mM potassium citrate), an iso-osmolar fluid commonly used for the storage of blood at 5°C. The blood was poured into the above-mentioned bags by means of a centrifuge which allowed the formed elements of the haematic mass to be separated from the erythrocytes. In addition to the above-mentioned CPDA-1, some of the bags contained L-carnitine, which was always introduced under conditions of maximum sterility. The ratio of the volume of the storage fluid to the volume of the erythrocytes was close to 1:1. The bags were then placed in refrigerators at a constant temperature of 5°C.

In the course of storage, aliquots of erythrocytes were collected at weekly intervals for the purposes of conducting a number of microscopic and biochemical examinations. The erythrocyte suspension was immediately examined under a phase-contrast microscope to estimate the percentage content of burr cells, which are pathological erythrocytes with a star-type morphology. Later, the erythrocyte suspension was centrifuged, and the buffy coat was used to determine the haemoglobin content according to a method involving the derivatiza-

tion of the latter to cyano-methaemoglobin (International Committee for Standardization in Haematology, S. Clin. Pathol. (1978), 31:139-145). The haemoglobin content measured is an indicator of the degree of haemolysis the erythrocyte undergoes in the course of storage in the bags. Lastly, erythrocyte osmotic fragility was evaluated according to the method of Dacie and Lewis (Dacie J.V. and Lewis S.M., Practical Haematology, New York Churchill Livingstone, 1984: 152-6). The osmotic fragility was calculated as the amount of sodium chloride necessary (in mM/L) to obtain 50% haemolysis.

Claims

1. Use of L-carnitine, alkanoyl L-carnitine and their pharmacologically acceptable salts for the production of stabilizing solutions for the storage of blood samples and blood component samples for transfusions.
2. The use of claim 1, wherein the blood component sample is an erythrocyte sample.
3. The use of claim 1, wherein the alkanoyl L-carnitine is selected from acetyl, propionyl, butyryl, isobutyryl, valeryl and isovaleryl L-carnitine.
4. A stabilizing solution for the storage of blood samples and blood component samples for transfusions, characterized in that it comprises:
 - (a) a conventional preservative-anticoagulant solution; and
 - (b) L-carnitine or an alkanoyl L-carnitine selected from acetyl, propionyl, butyryl, isobutyryl, valeryl and isovaleryl L-carnitine, or a pharmacologically acceptable salt thereof.
5. The stabilizing solution of claim 4, wherein the blood component sample is an erythrocyte sample.
6. The stabilizing solution of claim 4, wherein the conventional preservative-anticoagulant solution is selected from ACD, CPD and CPDA-1.
7. The stabilizing solution of claims 4-6, characterized in that it contains 0.5-10.0 mM/L, preferably 4-6 mM/L, of L-carnitine or of an alkanoyl L-carnitine or one of its pharmacologically acceptable salts.
8. The stabilizing solution of claim 7, characterized by the following composition:

| | |
|---------|-------------|
| Glucose | 80-120 mM/L |
|---------|-------------|

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(continued)

| | |
|---------------------------------|------------|
| Mannitol | 40-60 mM/L |
| K ₂ HPO ₄ | 24-28 mM/L |
| KH ₂ PO ₄ | 12-16 mM/L |
| Potassium citrate | 15-20 mM/L |
| L-carnitine, internal salt | 4-6 mM/L |

9. A transfusion bag which comprises:

- (a) a sample of blood or a sample of erythrocytes;
- (b) a conventional preservative-anticoagulant solution; and
- (c) 0.5-10.0 mM/L, preferably 4-6 mM/L, of L-carnitine or an alkanoyl L-carnitine selected from acetyl, propionyl, butyryl, isobutyryl, valeryl and isovaleryl L-carnitine, or a pharmacologically acceptable salt thereof.

10. The transfusion bag of claim 9, wherein the conventional preservative-anticoagulant solution is selected from ACD, CPD and CPDA-1.

Patentansprüche

1. Verwendung von L-Carnitin, Alkanoyl-L-Carnitin und deren pharmakologisch akzeptablen Salze für die Herstellung von Stabilisierlösungen für die Lagerung von Blutproben und Blutkomponentengruppen für Transfusionen.
2. Verwendung nach Anspruch 1, worin die Blutkomponentenprobe eine Erythrocytenprobe ist.
3. Verwendung nach Anspruch 1, worin das Alkanoyl-L-carnitin ausgewählt ist aus Acetyl-, Propionyl-, Butyryl-, Isobutyryl-, Valeryl- und Isovaleryl-L-carnitin.
4. Stabilisierlösung für die Lagerung von Blutproben und Komponentenproben für Transfusionen, dadurch gekennzeichnet, daß sie umfaßt:
 - (a) eine konventionelle Konservierungsmittel-Antikoagulans-Lösung; und
 - (b) L-Carnitin oder ein Alkanoyl-L-carnitin, ausgewählt aus Acetyl-, Propionyl-, Butyryl-, Isobutyryl-, Valeryl- und Isovaleryl-L-carnitin oder einem pharmakologisch akzeptablen Salz davon.
5. Stabilisierlösung nach Anspruch 4, worin die Blutkomponentenprobe eine Erythrocytenprobe ist.

6. Stabilisierlösung nach Anspruch 4, worin die konventionelle Konservierungsmittel-Antikoagulans-Lösung ausgewählt ist aus ACD, CPD und CPDA-1.

7. Stabilisierlösung nach den Ansprüchen 4 bis 6, dadurch gekennzeichnet, daß sie 0,5 bis 10,0 mM, bevorzugt 4-6 mM/L L-Carnitin oder Alkanoyl-L-carnitin oder eines seiner pharmakologisch akzeptablen Salze enthält.

8. Stabilisierlösung nach Anspruch 7, gekennzeichnet durch die folgende Zusammensetzung:

| | |
|---------------------------------|-------------|
| Glucose | 80-120 mM/L |
| Mannitol | 40-60 mM/L |
| K ₂ HPO ₄ | 24-28 mM/L |
| KH ₂ PO ₄ | 12-16 mM/L |
| Kaliumcitrat | 15-20 mM/L |
| Inneres Salz von L-Carnitin | 4-6 mM/L |

9. Transfusionsbeutel, umfassend:

- (a) eine Blutprobe oder eine Erythrocytenprobe;
- (b) eine konventionelle Konservierungsmittel-Antikoagulans-Lösung; und
- (c) 0,5 - 10,0 mM/L, bevorzugt 4-6 mM/L, L-Carnitin oder eines Alkanoyl-L-Carnitins, ausgewählt aus Acetyl-, Propionyl-, Butyryl-, Isobutyryl-, Valeryl- und Isovaleryl-L-carnitin, oder eines pharmakologisch akzeptablen Salzes davon.

10. Transfusionsbeutel nach Anspruch 9, worin die konventionelle Konservierungsmittel-Antikoagulans-Lösung ausgewählt ist aus ACD, CPD und CPDA-1.

Revendications

1. Utilisation de L-carnitine, d'un alkanoyl L-carnitine et de leurs sels pharmacologiquement acceptables, pour la production de solutions stabilisantes destinées à la conservation d'échantillons de sang et d'échantillons de composant sanguin pour transfusion.
2. Utilisation selon la revendication 1, dans laquelle l'échantillon de composant sanguin est un échantillon d'érythrocytes.

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3. Utilisation selon la revendication 1, dans laquelle l'alcanoyl L-carnitine est choisie parmi les acétyl, propionyl, butyryl, isobutyryl, valéryl et isovaléryl L-carnitines.

4. Solution stabilisante pour la conservation d'échantillons de sang et d'échantillons de composant sanguin pour transfusion, caractérisée en ce qu'elle comprend :

(a) une solution conservatrice et anticoagulante classique ; et

(b) de la L-carnitine ou une alcanoyl L-carnitine choisie parmi les acétyl, propionyl, butyryl, isobutyryl, valéryl et isovaléryl L-carnitines, ou un sel pharmacologiquement acceptable de celles-ci.

5. Solution stabilisante selon la revendication 4, dans laquelle l'échantillon de composant sanguin, est un échantillon d'érythrocytes.

6. Solution stabilisante selon la revendication 4, dans laquelle la solution conservatrice et anticoagulante classique est choisie parmi les solutions ACD, CPD et CPDA-1.

7. Solution stabilisante selon les revendications 4 à 6, caractérisée en ce qu'elle contient de 0,5 à 10,0 mmol/l, de préférence de 4 à 6 mmol/l de L-carnitine ou d'une alcanoyl L-carnitine, ou de l'un de ses sels pharmacologiquement acceptables.

8. Solution stabilisante selon la revendication 7, caractérisée par la composition suivante :

| | | |
|---------------------------------|---------------|----|
| Glucose | 80-120 mmol/l | 40 |
| Mannitol | 40-60 mmol/l | |
| K ₂ HPO ₄ | 24-28 mmol/l | |
| KH ₂ PO ₄ | 12-16 mmol/l | |
| Citrate de potassium | 15-20 mmol/l | 45 |
| Sel interne de L-carnitine | 4-6 mmol/l | |

9. Sac de transfusion comprenant :

(a) un échantillon de sang ou un échantillon d'érythrocytes ;

(b) une solution conservatrice et anticoagulante classique ; et

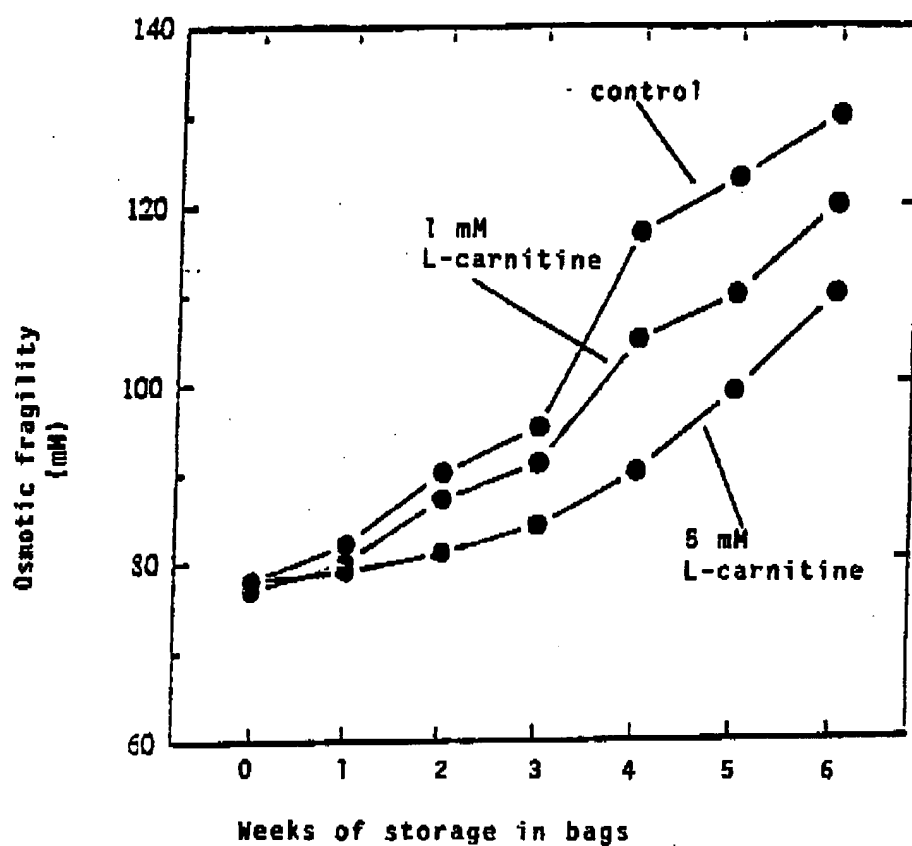
(c) de 0,5 à 10,0 mmol/l, de préférence de 4 à 6 mmol/l, de L-carnitine ou d'une alcanoyl L-carnitine choisie parmi les acétyl, propionyl, butyryl, isobutyryl, valéryl et isovaléryl L-carni-

tines, ou d'un sel pharmacologiquement acceptable de celles-ci.

10. Sac de transfusion selon la revendication 9, dans lequel la solution conservatrice et anticoagulante classique est choisie parmi les solutions ACD, CPD et CPDA-1.

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Fig. 1

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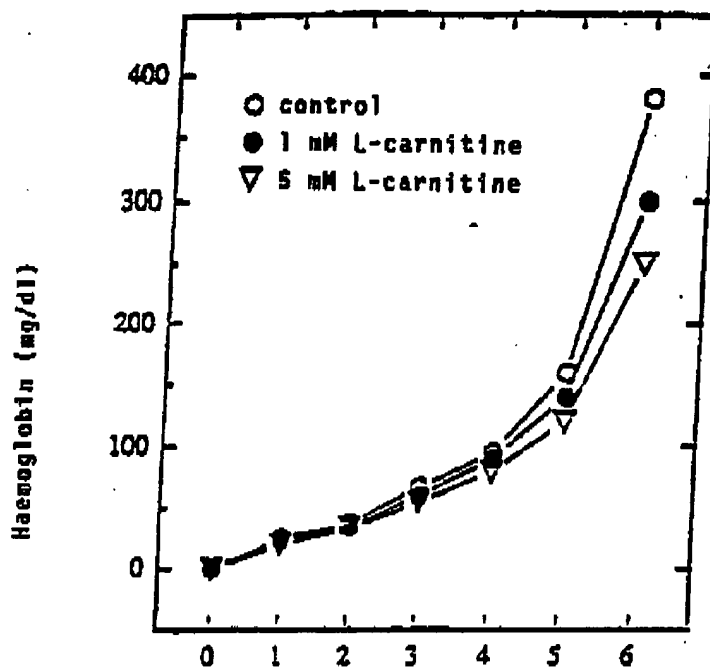


Fig. 2

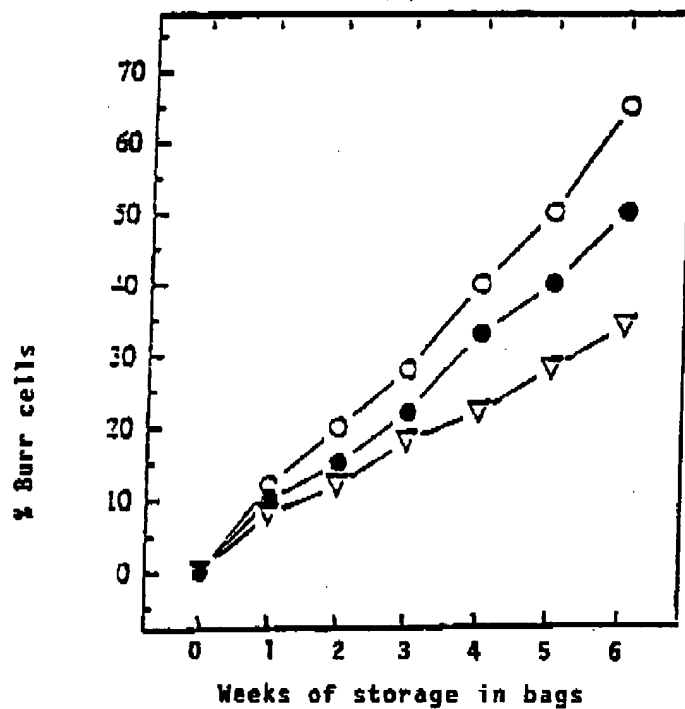


Fig. 3